

# Biomeme


# SARS-CoV-2

# Bulk Vials

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Version 1.2

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## Brief Overview

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Biomeme **SARS-CoV-2 Bulk Vial** detects the RNA of severe acute respiratory syndrome coronavirus 2 that causes coronavirus disease 2019 (COVID-19), also known as “2019-nCoV” or “Wuhan coronavirus.”

The Biomeme test detects two different SARS-CoV-2 genes and is multiplexed together with Biomeme's RNA Process Control (RPC) for RNA extraction and RT-PCR (MS2 bacteriophage). The SARS-CoV-2 Bulk Vial contains lyophilized master mix, enzymes, and multiplexed primer/probes for the following triplex reaction:

- **1ab** - Open reading frame 1ab gene
- **S** - Spike gene
- **RPC** - RNA Process Control (MS2 bacteriophage)

**Safety Warning:** When working with our products, always wear appropriate personal protective equipment (PPE) (e.g. lab coat, disposable gloves with adequate chemical resistance, mouth/face protection, goggles, etc.) For more information, please review the product's safety data sheet(s) (SDS).

# Contents of Bulk Vial Pouch

CONTENTS	DESCRIPTION
1x small clear bag	<p>The small clear bag includes:</p> <ul style="list-style-type: none"> <li>• 2x bulk vials. Each vial contains enough lyophilized assay for 65 reactions* (2 vials x 65 = 130 total reactions). The amount of loss will depend on the pipette used to resuspend the assay.</li> <li>• 2x screw cap tubes containing 675 uL of DNase/RNase free molecular grade water</li> <li>• 2x transfer pipettes</li> </ul> <p><b>NOTE:</b> The included disposable bulb pipette is sufficient to ensure at least 90 total reactions even with waste. Use a lab pipette to maximize your yield and achieve the maximum 130 reactions.</p>
1x small clear bag	<p>The small clear bag includes:</p> <ul style="list-style-type: none"> <li>• 1x small foil pouch with a screw cap tube containing a lyophilized pellet of quantified MS2 to be used as your RNA Process Control (RPC)</li> <li>• 1x screw cap tube containing 5 mL of pre-aliquoted RPC Buffer used to resuspend the lyophilized RPC pellet. 1x resuspended RPC is enough positive control for 250 sample extractions when adding 20 uL to each extraction.</li> <li>• 1x transfer pipette</li> </ul>

## Technical Characteristics

SPECIFICATIONS	VALUE
PCR Tube Capacity	0.1 mL
PCR Reaction Volume	20 µL
DNA-dependent DNA-polymerase	Hotstart Taq polymerase (1 min. activation @ 95°C)
Reverse transcriptase	Thermostable RNase H+ recombinant MMuLV (2 min. RT step @ 55°C)
Nucleotides	Proprietary mix of dNTPs

Buffer	Tris pH 8.8 Salts and enhancers for 5' nuclease assays
Mg <sup>++</sup>	6 mM
Storage	15-30°C
Dissolution time	~60s

**Note:** Contains Bovine Serum Albumin of USA origin. Certified BSE free

## Multiplex Assay Characteristics

Target	COLOR CHANNEL
SARS-CoV-2-Orf1ab gene	Green (FAM)
SARS-CoV-2-S gene	Red (ATTO647N)
RNA Process Control (Exogenous RNA Extraction and RT-PCR Process Control (MS2))	Amber (TexasRedX)

## Reconstitution Volume

ASSAY CONCENTRATION SOUGHT	DILUENT VOLUME TO ADD
2x	675 µL

# Prepare RNA Process Control (RPC)

Each Biomeme SARS-CoV-2 Test includes pouches containing your RNA Process Control (RPC), RPC Pellets (MS2 bacteriophage), and 1mL transfer pipettes.

1. Open a foil pouch and remove the screw cap tube containing your RPC pellet. Open tube.
2. Open the 5mL screw cap tube containing RPC buffer.
3. Using the 1mL transfer pipette, pull 0.5 - 0.75 mL of RPC buffer and add to the RPC pellet tube to resuspend.
4. Pipette up and down with the transfer pipette to mix.
5. Transfer the entire volume back into the 5 mL RPC buffer tube, again pipetting up and down to mix.
6. Your RPC is now ready to add to your upcoming sample extractions (when adding 20  $\mu$ L to the Biomeme extraction cartridge, this will equal ~400 pfu per 20  $\mu$ L PCR reaction).

*Once resuspended, the RPC Pellet can be refrigerated at 4 °C for up to one week. It can also be frozen at -20 °C but we caution against frequent freezing and re-thawing as it will degrade the control.*

7. Transfer 20  $\mu$ L of RPC to the lysis buffer of your sample extraction (the punctured red section of your sample prep cartridge if using Biomeme's M1 Sample Prep). If using the Biomeme 10  $\mu$ L fixed volume pipette kit, transfer 10  $\mu$ L twice (*remember to change pipette tips after each transfer*).
8. Close the RPC Buffer tube.

# Assay Setup

1. Using the 1 mL transfer pipette, add the entire volume (675  $\mu$ L) of included DNase/RNase free molecular grade water to the SARS-CoV-2 Bulk Vial to resuspend the assay at a 2x concentration.
2. Pipette the solution up and down several times or shake to mix. Discard the pipette and empty tube.
3. Add 10  $\mu$ L of assay into each of your PCR reaction wells. *Remember to change pipette tips each time you add your assay to a new well.* Each Bulk Vial contains enough assay for 65 reactions (without pipetting waste).
4. Transfer 10  $\mu$ L of eluate (containing purified nucleic acids) to each PCR reaction Biomeme's SARS-CoV-2 assay. 1 sample extraction per PCR reaction (not including any wells you may reserve for additional controls or replicates). *Remember to change pipette tips each time you add your purified sample to a new PCR reaction.*
5. Transfer your PCR reactions to your thermocycler and begin your run!

**Note:** For additional tips, How-To videos, and best practices for our Sample Prep system, please visit our Biomeme Sample Prep Guide, available at: <https://help.biomeme.com/sample-prep-guide>

# Thermocycling Parameters

		DURATION	
	TEMPERATURE (°C)	Biomeme Franklin™	Lab Thermocycler
<b>RT Step</b>	55	120 secs	120 secs
<b>Initial Denature</b>	95	60 secs	60 secs
<b>Cycling Denature</b>	95	3 secs	1 sec
<b>Annealing</b>	60	30 secs	20 secs
<b>Extension</b>	N/A	N/A	N/A
<b>Melt Curve</b>	N/A	N/A	N/A
<b>Number of Cycles: 45</b>		<b>Total Reaction Volume: 20 µL</b>	

**Note:** The above thermocycling parameters have been confirmed on the Bio-Rad CFX96, Applied Biosystems (ABI) 7500 Fast and/or QuantStudio5 using the “fast” block.

# In Silico Analysis

## ***Inclusivity:***

The SARS-CoV-2 primer and probe sets for both Orf1ab and S gene targets have 100% homology to published sequence from NCBI.

## ***Cross-reactivity (Exclusivity):***

The Orf1ab R primer returned 90% homology to SARS-CoV, however when primer BLAST was performed (which takes into account the Forward primer and the ability for PCR amplicon production) no target templates were found to SARS-CoV.

All other primers and probes had <80% homology to exclusive organisms listed below.

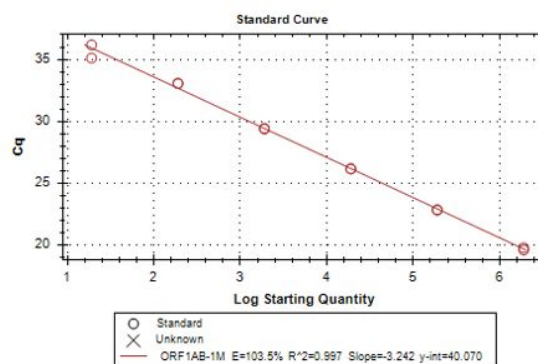
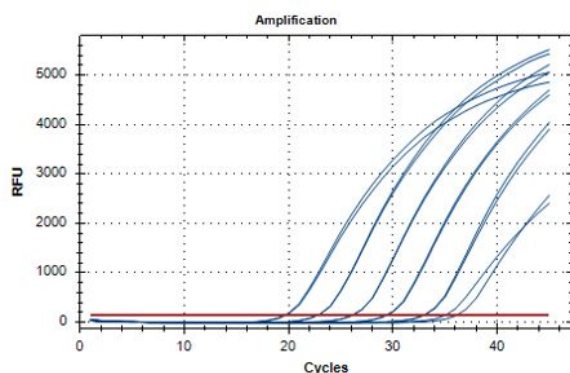
Human coronavirus 229E (taxid:11137)	Influenza A virus (taxid:11320)
Human coronavirus OC43 (taxid:31631)	Influenza B virus (taxid:11520)
Human coronavirus HKU1 (taxid:290028)	Human enterovirus A (taxid:138948)
Human coronavirus NL63 (taxid:277944)	Human enterovirus B (taxid:138949)
	Human enterovirus C (taxid:138950)
	Human respiratory syncytial virus (taxid:11250)
MERS coronavirus (taxid:1335626)	Rhinovirus (taxid:12059)
Human adenovirus B (taxid:108098)	Chlamydia pneumoniae (taxid:83558)
Human adenovirus D (taxid:130310)	Haemophilus influenzae (taxid:727)
Human adenovirus C (taxid:129951)	Legionella pneumophila (taxid:446)
Human adenovirus B1 (taxid:565302)	Mycobacterium tuberculosis (taxid:1773)
Human adenovirus 7 (taxid:10519)	Streptococcus pneumoniae (taxid:1313)
Human adenovirus B7 (taxid:10519)	Streptococcus pyogenes (taxid:1314)
Human adenovirus E (taxid:130308)	Bordetella pertussis (taxid:520)
Adenovirus type 8 (taxid:31545)	Mycoplasma pneumoniae (taxid:2104)
Human adenovirus F (taxid:130309)	Pneumocystis jiroveci (taxid:42068)
Human metapneumovirus (taxid:162145)	Candida albicans (taxid:5476)
Human parainfluenza virus 1 (taxid:12730)	Pseudomonas aeruginosa (taxid:287)
Human parainfluenza virus 2 (taxid:1979160)	Staphylococcus epidermidis (taxid:1282)
Human parainfluenza virus 3 (taxid:11216)	Staphylococcus group (taxid:90964)
Human parainfluenza virus 4 (taxid:1979161)	



# Performance Characteristics

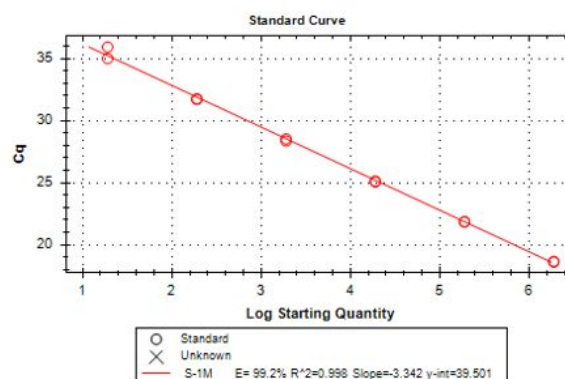
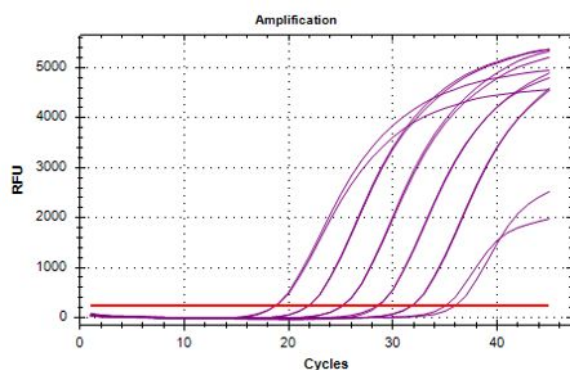
## **SARS-CoV-2 Orf1ab**

Amplification plots of multiplexed Orf1ab ivt-RNA in serial dilution from  $2 \times 10^6$  — 20 copies/reaction in lyophilized LyoRNA 2.0 Master Mix. Efficiency of 103.5%. Limit of detection not determined.



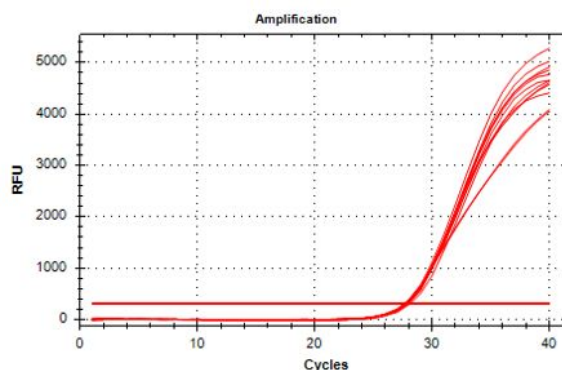
## **SARS-CoV-2 S**

Amplification plots of multiplexed S ivt-RNA in serial dilution from  $2 \times 10^6$  — 20 copies/reaction in lyophilized LyoRNA 2.0 Master Mix. Efficiency of 99.2%. Limit of detection not determined.



### ***RNA Process Control (Exogenous RNA Extraction and RT-PCR Control - MS2)***

Amplification of multiplexed MS2 RNA extraction and RT-PCR process control in lyophilized LyoRNA 2.0 Master Mix.



## **Storage**

Bulk Vials should be stored in a dry place, at room temperature (15-30°C). See the vial label for expiration date. After resuspension, use as quickly as possible and store at 4°C for up to eight hours

# Disclaimer

**For Research Use Only.** Not for use in human or veterinary diagnostics. The performance characteristics of this product have not been established.

Biomeme products may not be transferred to third parties, resold, modified for resale or used to manufacture commercial products or to provide a service to third parties without written approval of Biomeme, Inc.

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